

critical for binding. This is illustrated by the  $10^5$ - $10^6$  decrease in affinity on going from Ala-boroPro or Pro-boroPro to boroPro itself (Table 1).

The inhibition experiments presented in Table 1 were  
5 carried out on DP-IV isolated from pig kidneys. Pro-boroPro and Ala-boroPro inhibit DP-IV from human placenta equally well.

The Ala-boroPro and Pro-boroPro used in the experiments described above were racemic mixtures in which  
10 the boroPro moiety was present as both the D-form and L-form while Ala and Pro were both the L-isomer.

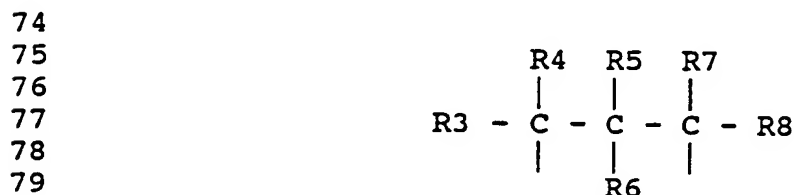
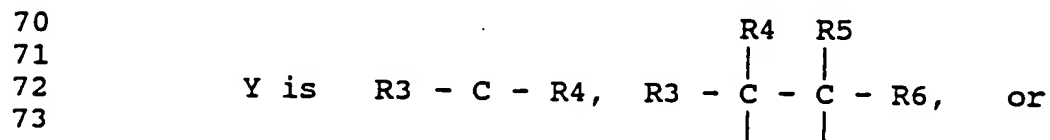
High pressure liquid chromatography (HPLC) can be used to separate L-Pro-D-boroPro from L-Pro-L-boroPro. A 4.6 mm x 250 mm Nucleosil C18 ( $5\mu$  particle) column employing  
15 a two buffer system (Buffer A is 100%  $H_2O$  with 0.1% TFA, and buffer B is 70%  $CH_3CN$ , 30%  $H_2O$ , 0.86% TFA) can be used to carry out the separation. From 0 to 5 min 5% B and 95% A is used, and from 5 to 25 min 5% to 100% B is used. The L,L isomer comes off first at about 7 min, followed by the L,D  
20 isomer at about 10 min. NMR and mass spectra analysis were consistent with both compounds being Pro-boroPro. Rechromatography of the purified isomers indicated that the first pass on the HPLC column achieved an isomeric purity of about 99-6% for each isomer. High pressure liquid  
25 chromatography (HPLC) can similarly be used to be used to separate L-Ala-D-boroPro from L-Ala-L-boroPro or to separate the D-boroPro form of other inhibitors from the L-boroPro form.

When L-Pro-L-boroPro and L-Pro-D-boroPro were used  
30 in a DP-IV inhibition assay, the  $K_i$  for L-Pro-L-boroPro was  $3.2 \times 10^{-11}M$ , while for L-Pro-D-boroPro the  $K_i$  was  $6.0 \times 10^{-8}M$ . The L,L-isomer constitutes a much better





where each J, independently, is O-alkyl, N-alkyl, or alkyl, each said O-alkyl, N-alkyl or alkyl comprising 1 - 20 carbon atoms and, optionally, heteroatoms which can be N, S, or O; said T being able to form a complex with the catalytic site of a dipeptidyl-aminopeptidase type IV (DP IV) enzyme;



and each R1, R2, R3, R4, R5, R6, R7, and R8, separately is a group which does not significantly interfere with site specific recognition of said inhibitory compound by said DP IV, and allows said complex to be formed with said DP IV.

2. The compound of claim 1, wherein T is a boronate group.

3. The compound of claim 1, wherein T is a phosphonate group or a trifluoroalkyl ketone group.

4. The compound of claim 1 wherein each R1 - R8 is H.

1           5. The compound of claim 1 or 2 wherein each R1 and  
2 R2 are H, and each Y is CH<sub>2</sub> - CH<sub>2</sub>.

1           6. The compound of claim 5 wherein each R is  
2 independently chosen from the R group of proline and  
3 alanine.

1           7. The compound of claim 1, wherein said compound  
2 has a binding or dissociation constant to said DP IV of at  
3 least 10<sup>-9</sup>M.

1           8. The compound of claim 1, wherein said compound  
2 has a binding constant to said DP IV of at least 10<sup>-8</sup>M.

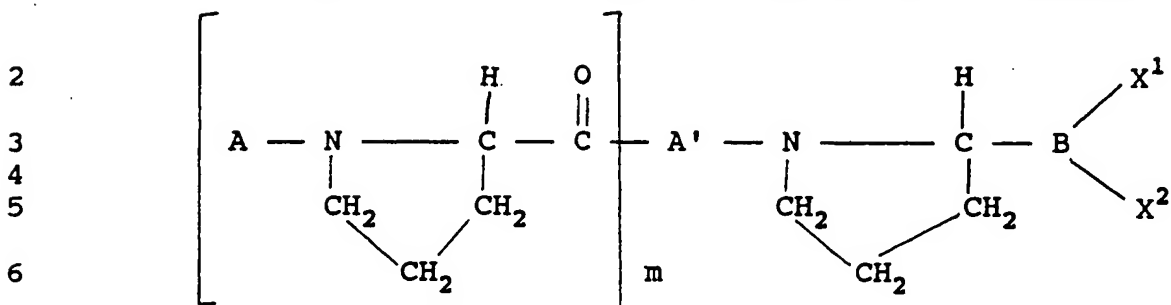
1           9. The compound of claim 1 admixed within a  
2 pharmaceutically acceptable carrier substance.

1           10. The compound of claim 1 wherein, each D1 and D2  
2 is, independently, F or D1 and D2 together are a ring  
3 containing 1 to about 20 carbon atoms, and optionally  
4 heteroatoms which can be N, S, or O.

1           11. A method for inhibiting DP IV in a mammal,  
2 comprising administering to said mammal an effective amount  
3 of a compound of claim 1.

1           12. The method of claim 11 wherein said amount is 1  
2 - 500 mg/kg/day.

1 13. An inhibitor of DP-IV, having the structure:



7 wherein m is an integer between 0 and 10, inclusive; A and  
8 A' are L-amino acid residues such that the A in each  
9 repeating bracketed unit can be a different amino acid  
10 residue; the C bonded to B is in the L-configuration; the  
11 bonds between A and N, A and C, and between A and N are  
12 peptide bonds; and each X<sup>1</sup> and X<sup>2</sup> is, independently, a  
13 hydroxyl group or a group capable of being hydrolysed to a  
14 hydroxyl group at physiological pH.

1 14. The inhibitor of claim 13 wherein A and A' are  
2 independently proline or alanine residues.

1 15. The inhibitor of claim 13 wherein m is 0.

1 16. The inhibitor of claim 13 wherein X<sup>1</sup> and X<sup>2</sup> are  
2 hydroxyl groups.

1 17. The inhibitor of claim 13 wherein said  
2 inhibitor is L-Ala-L-boroPro.

1 18. The inhibitor of claim 13 wherein said  
2 inhibitor is L-Pro-L-boroPro.

1           19. A method for inhibiting DP-IV in a mammal,  
2 comprising administering to said mammal an effective amount  
3 of a compound of claim 13.

1           20. The method of claim 19 wherein said amount is  
2 1 mg/kg of said mammal per day to 500 mg/kg of said mammal  
3 per day.